PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference HY 2 PCT	**FOR FURTHER ACTION** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/FI 03/00776	International filing date *(day/month/year)* 17.10.2003	Priority date *(day/month/year)* 21.10.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/12		
Applicant RNA-LINE OY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13.05.2004	07.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Scheffzyk-Sonnauer, Telephone No. +49 89 2399-8602

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/FI 03/00776

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-56 as originally filed

Sequence listings part of the description, Pages

1-18 as originally filed

Claims, Numbers

1-40 received on 17.11.2004 with letter of 12.11.2004

Drawings, Sheets

1/12-12/12 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 13

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 13

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-12,14-40
	No:	Claims	1-7
Inventive step (IS)	Yes:	Claims	23-28, 35-39
	No:	Claims	1-12, 14-22, 29, 30-34, 40
Industrial applicability (IA)	Yes:	Claims	1-12, 14-40
	No:	Claims	

2. Citations and explanations

   see separate sheet

SECTION V----------

Applicant's comments have been carefully considered but are not deemed persuasive.

Due to the broad scope of claims 1-7 these claims are foreshadowed by the teachings of US 2001/0023067 (1) (see page 2, left col. paragraph 13 from which the claimed method is implicitely derivable since without performing such a method there would not be the knowledge concerning the ability outlined in said paragraph) and Schiebel et al., The Journal of Biological Chemistry, vol. 263, no. 16, 1993, pp. 11851-11857 (2) (please see section "assay for RNA polymerase"). It is true that none of the available documents expressly mentions "scattered throughout the entire template length. However, for the time being said expression is not deemed suitable to render the claimed subject-matter clearly and unambiguously novel over the prior art since first one cannot rule out that said requirement is an intrinsic feature of for instance the polymerases taught in (1) and (2) and secondly because of the unclear meaning of "capable of" (see below).

Furthermore, in view of the disclosure of (3) and Genes and Development 16:790-795 (4) (see e.g. abstract) the subject-matter of claims 1-12,14-22, 29, 30-34 and 40 cannot be considered to be inventive (Art. 33(3) PCT).

Claims 23-28 and 35-39 seem to be novel and inventive (provided that the term "capable of" is read in the sense that the polymerase has intrinsically the required activity,; i.e. no modifications thereof are necessary- see below) since the fragment and the activity thereof is neither taught nor suggested in the available prior art.

Additional remarks:

1). With respect to the terms "short" and "sufficient" objections for lack of clarity arise since these terms are subjective and hence open for interpretation. Moreover, a claim must be clear when seen alone, i.e. in the absence of the description.

2). With respect to claims which are not limited to the QDE-1 protein objections under Art. 5/6 PCT arise since present application only deals with said QDE-1 protein but is completely silent with respect to the existence of other RNA polymerases

isolated from other organisms. Relating to this this Authority would like to emphasize that the scope of a claim basically should correspond to the technical contribution made by an application.

3). The term "capable of" renders the scope of claims containing said expression ambiguous since it is unclear whether the poymerase exhibits the required property, i.e. the production of short complementary RNA copies of a template scattered throughout the entire template length intrinsically or whether some modifications thereof which would be suitable to impart said property to a polymerase are also allowed by said term.

4). The category of claim 40 is unclear.

CLAIMS

1. A method for producing a nucleic acid product, comprising that an isolated polymerase protein is contacted with a nucleic acid template under conditions sufficient for the function of the enzyme, and wherein said polymerase protein is an RNA polymerase capable of producing

(a) short complementary RNA copies of said template, which are scattered throughout the entire template length and, optionally,

(b) template-length complementary RNA copies.

2. The method according to claim 1, wherein said nucleic acid template is DNA or RNA.

3. The method according to claim 1, wherein the ratio of said short and template-length RNA copies can be adjusted by the reaction conditions.

4. The method according to claim 1, wherein the length of said short RNA copies can be adjusted by the reaction conditions.

5. The method according to claim 1, wherein the said short or template-length RNA copies are annealed to the template or denatured from the template.

6. The method according to claim 1, wherein said nucleic acid template is linear or circular.

7. The method according to claim 1, wherein said polymerase originates from a eukaryotic cell.

8. The method according to claim 7, wherein said polymerase originates from an organism selected from the kingdoms of Fungi, Viridiplantae, Metazoa, or the group of Mycetozoa.

9. The method according to claims 7 or 8, wherein said polymerase originates from an organism selected from the subset of genera *Neurospora*, *Arabidopsis*, *Caenorhabditis*, and *Dictyostelium*, preferebly organisms *Neurospora crassa*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, and *Dictyostelium discoideum*.

10. The method according to claim 9, wherein said polymerase is QDE-1 protein of *Neurospora crassa* or an altered or a genetically modified derivative of QDE-1.

11. The method according to any one of the preceding claims, wherein said RNA polymerase is encoded by a nucleic acid sequence selected from the group of:

(a) a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3;

(b) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO 2 or SEQ ID NO: 4;

(c) a nucleic acid sequence, which differs from the nucleic acid sequence of (a) or (b) due to degeneracy of the genetic code;

(d) a nucleic acid sequence hybridizing to the nucleic acid sequence of (a), (b) and/or (c) under stringent conditions; and

(e) a nucleic acid sequence encoding a polypeptide comprising the amino acids 709 to 1402 of SEQ ID NO:4 or any sequence longer than that up to the sequence comprising the amino acids 2 to 1402 of SEQ ID NO: 2; and

(f) a nucleic acid sequence encoding an amino acid sequence, which shows at least 50% identity to the amino acid sequence of SEQ ID NO: 2.

12. The method according to claim 1, comprising the steps of:

(a) providing ssRNA or ssDNA template;

(b) contacting said ssRNA or ssDNA template *in vitro* with the polymerase under conditions sufficient for RNA synthesis.

13. The method according to claim 12, wherein said ssRNA template is provided by transcribing a DNA template with a DNA-dependent RNA polymerase, preferably polymerase selected from the group of DNA bacteriophage-encoded DNA-dependent RNA polymerases, most preferably DNA-dependent RNA polymerase of bacteriophage T7, T3 or SP6.

14. The method according to claims 12 or 13, wherein the reactions are carried out at the same time or sequentially in the same reaction vessel.

15. The method according to any one of claims 12 to 14, wherein the newly produced RNA species are recovered from the reaction mixture.

16. The method according to any one of claims 12 to 15, wherein said newly produced RNA strands are annealed with the template to form dsRNA elements or, alternatively, are denatured from the template.

17. The method according to any one of claims 12 to 16, wherein RNA synthesis is initiated from the 3' end of a nucleic acid primer complementary to the RNA or DNA template or RNA synthesis is initiated without a primer.

18. The method according to any one of claims 12 to 17, wherein the reaction mixture for RNA synthesis comprises at least one nucleoside triphosphate optionally labeled with a radioactive isotope or is chemically modified, pH buffer, ammonium acetate, PEG, Mg^{2+} ions, Mn^{2+} ions and/or non-ionic detergent.

19. The method according to claim 18, wherein the method is specifically used for producing radioactively or chemically labeled RNA probes and comprising an optional step of purifying the newly produced labeled RNA from the components of the reaction mixture.

20. The method according to claim 19, wherein said labeled RNAs are used as probes for Southern or Northern blot analyses after the optional purification step.

21. The method according to claim 19, wherein said labeled RNAs are used as probes for a fluorescent *in situ* hybridization analysis after the optional purification step.

22. The method according to claim 19, wherein said labeled RNAs are used as probes for a microarray analysis after the optional purification step.

23. An isolated polypeptide, characterized in that:

(i) said polypeptide has sufficient RNA polymerase activity and is capable, when contacted with a nucleic acid template, of producing short complementary

RNA copies of said template, which RNA copies are scattered throughout the entire template length and, optionally, capable of producing template-length complementary RNA copies;

(ii) said polypeptide has enhanced solubility resulting in at least 3 times higher yield of the active polymerase, than in the case of polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or encoded by the nucleic acid sequence comprising SEQ ID NO: 1; and

(iii) said polypeptide is encoded by a nucleic acid sequence selected from the group of:

(a) a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 3;

(b) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 4;

(c) a nucleic acid sequence, which differs from the nucleic acid sequence of (a) or (b) due to degeneracy of the genetic code;

(d) a nucleic acid sequence hybridizing to the nucleic acid sequence of (a), (b) and/or (c) under stringent conditions;

(e) a nucleic acid sequence encoding a polypeptide comprising the amino acids 709 to 1402 of SEQ ID NO:4 or any sequence longer than that up to the sequence comprising the amino acids 2 to 1402 of SEQ ID NO: 2; and

(f) a nucleic acid sequence encoding an amino acid sequence, which shows at least 50% identity to the amino acid sequence SEQ ID NO: 2.

24. An isolated nucleic acid sequence encoding the polypeptide according to claim 23.

25. A vector, which comprises the nucleic acid sequence of claim 24 operationally linked with regulatory sequences required for gene expression.

26. A host cell comprising the vector of claim 25.

27. A method for producing a polymerase protein, which comprises culturing the host cell of claim 26 under conditions suitable for the expression of the protein.

28. The method according to claim 27, comprising the step that the protein is recovered from the cell or culture medium and optionally purified.

29. A method for studying nucleic acid secondary structure, preferably RNA secondary structure comprising the steps of:

(a) providing nucleic acid target molecule, preferably RNA target molecule;

(b) contacting said target molecule with an isolated RNA polymerase capable of producing short complementary RNA copies of the nucleic acid target as template, which RNA copies are scattered throughout the ssRNA regions of the template length and, optionally, template-length complementary RNA copies under conditions sufficient for RNA synthesis in a mixture additionally comprising radioactively or chemically labeled nucleotides, so that single-stranded elements of said target RNA are copied by the polymerase;

(c) recovering and optionally purifying the newly produced labeled nucleic acid species from the reaction mixture;

(d) using said labeled nucleic acid species as probes for microarray chip that comprises nucleic acid fragments of said target molecule;.

(e) interpreting data from the microarray analysis to deduce which parts of the target molecule are single-stranded; and optionally

(f) building a model for the secondary or tertiary structure of the target molecule.

30. A method for studying nucleic acid-protein interactions, preferably RNA-protein interactions comprising the steps of:

(a) providing a nucleic acid target and nucleic acid binding protein, preferably an RNA target and an RNA-binding protein;

(b) contacting said target and the solution of said protein in an experimental mixture under conditions sufficient for target-protein interaction, and in a separate vessel, contacting said target with a control solution that lacks said protein.

(c) contacting said experimental and control mixtures with an isolated RNA polymerase capable of producing short complementary RNA copies of said target as template, which RNA copies are scattered throughout the template length not

covered by protein and, optionally, template-length complementary RNA copies under conditions sufficient for RNA synthesis;

(d) recovering and optionally purifying the newly produced labeled nucleic acid species from both reaction mixtures;

(e) using the two sets of labeled nucleic acid species as probes for two identical microarray chips that comprise nucleic acid fragments of the target;

(f) interpreting data from the two microarray analyses to deduce which parts of the target molecule are accessible for the RNA synthesis;

(g) comparing the two data sets to determine the difference between target in experimental and control mixtures; and optionally

(h) interpreting the difference between the two data sets as a model for nucleic acid-protein interactions

31. A method for producing RNA trigger molecules to induce RNA interference *in vivo* or *in vitro*, comprising the steps of:

(a) providing RNA or DNA template;

(b) contacting said RNA or DNA template with an isolated RNA polymerase capable of producing short complementary RNA copies of said template, which are scattered throughout the entire template length and, optionally, template-length complementary RNA copies under conditions sufficient for RNA synthesis in a mixture comprising: nucleic acid template, said RNA polymerase, nucleoside triphosphates, and optionally pH buffer, ammonium acetate, PEG, Mg^{2+} ions, Mn^{2+} ions and/or non-ionic detergent; and

(c) incubating the reaction mixture at temperature sufficient for RNA synthesis.

32. The method according to claim 31, wherein said RNA or DNA template originates from a cell or a virus.

33. The method according to claim 31, wherein said RNA template is provided by transcribing a DNA template with a DNA-dependent RNA polymerase, preferably derived from a bacteriophage selected from the group of T7, T3, and SP6 bacteriophages.

34. The method according to claim 31 or 33, wherein steps (a) and (b) are carried out at the same time or sequentially in the same reaction vessel.

35. A kit comprising the polypeptide of claim 23.

36. The kit according to claim 35, wherein the kit further comprises additives necessary for a detectable level of RNA synthesis.

37. The kit according to claims 35 or 36 comprising nucleoside triphosphates in concentrations sufficient for RNA synthesis.

38. The kit according to any one of claims 35 to 37, wherein at least one nucleoside triphosphate is labeled with a radioactive isotope or is chemically modified.

39. The kit according to any one of claims 35 to 38, additionally comprising a standard nucleic acid preparation (or preparations) with characterized capacity to serve as a template (templates) for RNA synthesis by the polypeptide of claim 23.

40. Use of the method of claim 1 for studying nucleic acid secondary structure, for studying nucleic acid-protein interactions, or for producing RNA trigger molecules.